FURTHER BIOLOGICAL NOTES ON THE COLORADO POTATO BEETLE, LEPTINOTARSA DECEMLINEATA (SAY), INCLUDING OBSERVATIONS ON THE NUMBER OF GENERATIONS AND LENGTH OF THE PERIOD OF OVIPOSITION.

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During 1907 I have tried to duplicate the observations on this insect made in Georgia in 1906 (Girault and Rosenfeld, 1907), and also to extend them in length of time, and in a measure have succeeded in making some interesting and rather important ones on its life history. These observations were made during time not otherwise employed with the duties connected with a field station, and hence they are not by any means as complete and extensive as they should be and are necessarily more or less desultory; they were also started somewhat late in the season. But notwithstanding these, the facts learned I consider of importance, particularly as they tend to supplement some of the results obtained by Tower (1906), which are not very well known to entomologists. Not enough observations were made in most cases to warrant final conclusions, and with this general warning they are submitted for publication.

The observations were made in the field laboratory of the Bureau of Entomology, U. S. Department of Agriculture, at New Richmond, Clermont County, Ohio, latitude 38 degrees, 48 minutes, north.

SUMMARY.

The following paper is based on observations made during a single season on two pairs of hibernated beetles, interbred to a second generation which reproduced, contrary to the results obtained by Tower (1906) for normal beetles. A record of oviposition is given for each generation, in the case of the hibernated pairs exceeding the average recorded by Tower for normal beetles. The length of life of the adults and the period of oviposition exceeds in each instance the average recorded by the same author. In addition to observations on these points, a number of records of the duration of the egg instar for different dates are given, together with observations on habits of the larvae and adults, and a few records of the duration of the post-embryonic instars.

The paper mainly deals, however, with observations on the adult, and especially on the number of generations per season and related points concerned with the function of reproduction, and the interpretation of these observations in the light of the recent work of Tower (1906). I have, therefore, freely interspersed quotations from this work, and discussed somewhat at length the evidence so far presented in regard to the number of cycles per season, reaching the general conclusion that my observations are indeterminate in value as far as this point is concerned, mainly because of paucity; that they tend, however, to throw the question open again, especially as Tower gives but a limited amount of evidence, and that the question is entirely undecided as far as out-door conditions are concerned. I have seen no valid reason to doubt Tower's statements, excepting that the data back of them have been withheld, and that unfortunate discrepancies arise from place to place throughout.

TABLE I.—Duration of the Egg Instar, New Richmond, Ohio, Season 1907.

Remarks.*	Lot No.	No. Eggs.	Deposited.			Hatched.			Duration.		Effective temp. Degrees Fahr. Daily Average.
			Mnoth.	Day.	Time.	Month.	Day.	Time.	Days.	Hours.	
	1	44	May	31	2 pm	June	10	6 am	9	16	22.42
	2	58	June	1	9 pm		11	9 am	9	12	23.62
Pair No 1	3	62		4	9 am		12	noon	8	3	23.72
	4	52		9	10 am		15	noon	6	2	27.14
Pair No. 1	5	30		12	3 pm		18	5 pm	6	2	28.32
Pair No. 1	6	10		18	1 pm		23	5 am	4	16	33.85
	7	36		18	4 pm		23	7 am	4	15	33.85
	8	30		20	1 pm		25	5 am	4	16	34.94
	9	44		24	11 am		29	1 pm	5	2	30.70
	10	29	July	5	5 am	July	9	6 am	4	1	35.75
	11	33		7	noon		11	4 pm	4	4	37.16
	12	41		8	noon		12	10 pm	4	10	36.56
Pair No. 1	13	33		12	12:30 pm		16	11:30 pm	3	23	33.20
	14	31		19	1:30 pm		23	10:30 am	3	22	38.94
Pair No. 1, 1st generation.	15	31		23	6 pm		28	6 am	4	12	
Pair No. 1 1st generation.	16	22		28	2 pm	Aug.	2	10 am	4	20	31.94
Pair No. 1 1st generation.	17	21		29	1 pm		3	noon	4	23	32.38
Pair No. 1	18	7	Aug.	7	1 pm		11	8 pm	4	7	
Pair No. 1	19	28		13	7 pm		18	3 pm	4	20	
Pair No. 1	20	20		20	noon		25	4 am	4	16	
Lot No. 2 2nd generation.	21	39	Sept.	4	3 pm	Sept.	10	noon	5	21†	28.28†
Lot No. 1 2nd generation.	22	9		5	noon		11	6 am	5	18†	
Sums	22	710							107	281	532.77
Averages									5.40		31.34

* These refer to the hibernated pairs unless otherwise stated; and unless noted, all of these eggs are from pair No. 2 of the hibernated individuals.
† Approximated.

THE EGG.

1. *Length of Instar.* The duration of the egg instar has been determined for over seven hundred cases at different dates during the breeding season and the records are tabulated in table I. Each separate batch of eggs was confined immediately after deposition in an ordinary pasteboard pill-box, and they were thus in darkness. Moisture was supplied by the daily addition of fresh foliage. The temperature was the most apparent variable factor during development.

The effective temperatures were not determined in five cases, but enough are recorded to show that during the period covered the instar is about inversely proportional to the variation in temperature; that is to say, when the instar is long, the daily average effective temperature is low, and conversely. These records should have been made from a thermograph and calculated on the basis of hours; instead, I had to depend on maxima and minima for daily averages, and hence fractions of days had to be largely ignored. Undoubtedly, therefore, the records of effective temperatures in table I are more or less inaccurate. Forty-three degrees Fahrenheit is here assumed to mark the point of the inception of activity and reproduction, which as yet has not been determined for decemlineata. Wheeler (1889, p. 355) records the period of embryonic development as 6 days.

2. *Number of Eggs Deposited.* Our ignorance in regard to the average reproductive capability of our most common insects is profound, and this fact is well illustrated in the case of the Colorado Potato Beetle. I have simply to point out the fact that up to within the past two or three years its maximum oviposition was supposed to be in the neighborhood of 500 eggs, more probably less than that number. My observations on this point in 1907 are of importance because they show that this *estimate* is wrong, and also that with how slight an effort many of the facts of this nature can be learned more or less definitely. It was unfortunate that with the time at my disposal I was unable to make a large series of observations, thus obtaining maxima, minima, range, and average. William Lawrence Tower (1906) was the first to record actual observations on this point; apparently from a number of observations, on page 237, table 104, he records a range in oviposition of from 190 to 600 eggs, with an average of 450. The number of batches of eggs deposited ranged from 4 to 18 with an average of 12. Later (July 23, 1907,

in litt.), he states: "In regard to the number of eggs, on page 237 of my paper, you will find a table with the number of eggs laid by fifteen species of Leptinotarsa. In nature the average for decemlineata is 450—with a range of from 34 to 3700. I now have a female which has been laying eggs since April 12, and now has laid nearly 3,000 eggs. As she is in good condition, I expect to get from 3,500 to 4,000 eggs from her. This is a special race but shows the great range and the length of life of the adults. The reproductive period is in this race from 3 to 4 months long." It must not be understood from this quotation that the range of oviposition of from 34 to 3,700 as there stated is given in the place referred to (namely, Tower, 1906, p. 237, table 104), and I have since received the following from Professor Tower in regard to that statement (Tower, *in litt.*, April 21, 1908): "In regard to the number of eggs laid by Leptinotarsa decemlineata, the figures which I gave you last July were from unpublished data. The data to which you refer in my paper of 1906 are quite incomplete in regard to a lot of biological data which I did not feel like publishing at that time. There are some races of various species of Leptinotarsa which have laid eggs far in excess of 3,700, but these of course are special races. There was no mistake in giving the data. I simply gave you data which was unpublished."

Professor Tower has therefore obtained races which produce eggs far in excess of the normal beetles and which also have a much longer period of reproduction. The following account, of course, concerns normal beetles only, and so far as it goes, exceeds the averages obtained by Tower (1906) as far as reported for normal beetles.

Inasmuch as the period of oviposition and number of eggs deposited varied with the generation, the following table has been prepared showing the results obtained with the few pairs I was able to care for. Unfortunately, I was unable to keep a parallel series under field or natural conditions. This table also contains a number of observations closely connected with the function of oviposition, but difficult to present in any other form.

The table shows quite a range in oviposition according to generation, but as I have already indicated the observations are on too small a scale to allow general conclusions.

The first two pairs of hibernated adults were captured while mating in a potato field near New Richmond, Ohio, at 4 P. M., May 29, 1907, and each immediately confined in a glass jar cov-

TABLE II. NUMBER EGGS DEPOSITED IN CONFINEMENT BY PAIRS OF DIFFERENT GENERATIONS. OHIO. 1907.

Mass No.	Generation: Hibernated (Parents of 1st generation)				Generation: I (Parents of 2nd generation)				Generation: II (Parents of 3rd generation)			
	1st mating observed May 29, 4 p. m.				1st mating observed July 22, 10 a. m.				1st mating observed Sept. 1.			
	Pair No. 1		Pair No. 2		Pair No. 1		Pair No. 2		Lot No. 1		Lot. No. 2	
	Date	No. eggs	Date	No. eggs	Date	No. eggs	Date	No. eggs	Date	No. eggs	Date	No. eggs
1	June am 1	91	May 2 pm 31	49	July 3:30pm22	7	July 27 by pm	10	Sept. 4	6	Sept. 2	2
2	9 am 4	28	June 9 pm 1	58	6 pm 23	31			noon 5	16	3 pm 4	39
3		34	10 am 6	68	by pm 27	156			7	9	am 6	24
4	noon 5	20	10 am 9	52	2 pm 28	22						
5	11 am 6	9	11	20	1 pm 29	21						
6	pm 8	8	am 12	61	1 pm 30	28						
7	pm 9	43	14	21	pm 31	16						
8	2 pm 10	14	pm 16	17	Aug. am 3	20						
9	3 pm 12	30	am 18	17	am 3	26						
10	3 pm 15	33	4 pm 18	36	pm 4	27						
11	1 pm 17	6	pm 19	21	pm 6	17						
12	1 pm 18	10	1 pm 20	31								
13	am 19	21	21	71								
14	am 20	18	pm 22	48								
15	21	7	23	29								
16	23	41	11 am 24	44								
17	pm 24	12	26	46								
18	10 am 25	26	27	33								
19	26	31	pm 28	36								
20	27	18	July am 2	58								
21	30	6	pm 3	19								
22	July 4	15	am 5	29								
23	6	19	pm 5	26								
24	am 7	23	4 pm 6	35								
25	am 8	40	noon 7	33								
26	pm 8	16	noon 8	41								
27	By pm 11	60	am 9	18								
28	12.30 „ 12	33	by pm 11	41								
29	by am 14	13	am 16	22								
30	pm 15	14	pm 18	24								
31	pm 17	28	1:30pm19	31								
32	pm 18	13	pm 20	31								
33	pm 20	48	pm 21	11								
34	pm 21	17	pm 23	12								
35	11 am 22	23										
36	pm 23	25										
37	By 27	1										
38	Aug. am 2	11										
39	am 6	14										
40	pm 6	12										
41	1 pm 7	7										
42	pm 10	23										
43	am 11	14										
44	am 13	10										
45	7 pm 13	28										
46	By4pm16	10										
47	By am 19	1										
48	noon 20	20										
49	pm 23	51										
50	2 pm 24	2										
	Total	1097	Total	1189	Total	371	Total	10	Total	31	Total	65
	Average: 1143 eggs.				Average: 190.5 eggs.				Average: 48 eggs.			
	No. batches 50 Av. per batch 21.94 Daily average 12.90		No. batches, 34 Av. per batch. 34.97 Daily average 22.01		No. batches, 11 Av. per batch 33.72 Daily average 24.73		No. batches, 1 Av. per batch 10 Daily average 10		No. batches, 3 Av. per batch 10.3 Daily average 10.3		No. batches, 3 Av. per batch 21.6 Daily average 16.25	

ered with cheese-cloth and containing fresh soil and potato foliage. They were kept in the shade on a counter in the laboratory, and supplied fresh foliage daily. The pairs of the first generation are direct descendants of pair No. 2 of the hibernated pairs, and the two lots of the second generation are descendants of pair No. 1 of the first generation (*Vide seq.*, history of generations). These later pairs were confined in a manner similar to the first two, in the first generation, all of the individuals together until mated, and in the second generation the pairs were not separated from the whole lot.

It is clear from the table that the hibernated pairs deposited very many more eggs than did the pairs of the first generation, and in proportion, the first generation many more than did the pairs of the second. The average number of eggs deposited by each generation is given in the table; the average for the three groups of pairs is 460.5 eggs, but this has no relation with Tower's (1906) average of 450 eggs which I believe is intended as the average of any one pair in any one generation. The number of eggs and the number of batches of eggs deposited by the pairs of the hibernated individuals is the largest ever recorded for normal beetles, the latter ranging from 34 to 50, with an average of 42. Tower (1906, l. c.) records the range in batches of eggs to be from 4 to 18, with an average of 12. The average number of eggs per batch or mass ranges from 10 to 34.94 eggs, and the daily rate of oviposition from 10 to 24.73 eggs.

3. *Color of the Eggs First Deposited.* It may be mentioned in passing that the first eggs deposited by the pairs of the first generation were distinctly more reddish than usual; in pair No. 1 not becoming normally colored until the third mass was deposited (July 24th to 27th), and with Pair No. 2, the one mass of eggs deposited was of reddish hue, and the same was true of all of the eggs deposited by adults of the second generation. The coloring matter in the first eggs deposited appears to be richer, and becomes faded somewhat or less dense in the later eggs which are probably more mature before leaving the ovaries. A partial explanation of this is apparently given by Wheeler (1889). All of these eggs were fertile. On September 6th, a recently deposited mass of 29 eggs found in the field were of the same color and proved to be fertile.

THE LARVA.

1. *Length of Instars.* Observations on this point were not extended owing to the fact that it was impossible to make them continuous in time. Larvae hatching from 49 eggs (deposited at 2 P. M., May 31st, by pair No. 2 of the hibernated individuals) at 6 A. M., June 10th, molted for the first time at 9 P. M., June 15th; the time of other ecdyses not recorded. Forty-four larvae hatching at 5 A. M., June 25th, molted for the first time at 6 A. M., June 28th, and for the second time at 7 A. M., July 1, but the time of the third ecdysis was not recorded. The following table shows the only complete observation made on the duration of the larval instars.

TABLE III. DURATION OF LARVAL INSTARS FOR A SINGLE CYCLE. 1907.

Lot No.	No. larvae.	Hatched.	1st ecdysis Instar I.	2nd ecdysis Instar II	3rd ecdysis Instar III	Entered soil Instar IV	Sums Days.	Sums Hours	Total effective temperature. Degrees Fahr.
1	13	8 a. m. Aug. 30.	11 p. m. Sept. 1	4 p. m. Sept. 4	3 p. m. Sept. 8	5 p. m. Sept. 11			
			2 da., 15 hr.	2 da., 17 hr.	3 da., 23 hr.	3 da., 2 hr.	12	9	387.5°

2. *Number of Ecdyses.* The number of ecdyses recorded for fifty larvae during the season of 1907 was three (3), excluding pupation, and there were thus four distinct larval instars (*Vide* Girault, 1907).

3. *Length of Stage.* The duration of the larval stage was correctly determined for a few lots only; the observations on several others being interrupted at critical periods, thus destroying accuracy. The accompanying table summarizes.

TABLE IV. DURATION OF THE LARVAL STAGE FOR THREE CYCLES, 1907.

Lot No.	No. larvae.	Source.	Hatched.	Entered soil.	Duration Days	Duration Hours	Average daily effective temp. degrees Fahr.
1	30	Pair No. 2. Hibernated pairs.	5 a. m., June 25.	6 p. m., July 6.	11	13	30.8
2	21	Pair No. 1, 1st generation.	Noon, August 3.	10 a. m., Aug. 14.	10	22	32.0
3	13	Pair No. 1, Hibernated pairs.	8 a. m., Aug. 30.	5 p. m., Sept. 11.	12	9	29.8°

4. *Habits; Eating of Eggs in Nature by Young Larvae.* On September 6th, 1907, I observed a mass of 32 eggs on a potato plant in the field. These eggs were in the process of hatching, ten larvae having already excluded, and the remaining embryos being on the point of doing so. The ten very recently hatched larvae were busily making their first meals on the remaining unhatched eggs in the mass, nearly all of the perfect embryos in them having already been killed. The potato plants were in good condition, though it was rather late, and breeding was still in progress in this field; an abundance of food was at hand. This habit was previously observed in confinement (Girault and Rosenfeld, 1907, p. 53), and was then attributed to starvation. As yet it is impossible to say to what extent this occurs.

5. *Length of Life of Instar I, in Confinement without Food.* Three lots of larvae, during the second and third weeks in June, were allowed to hatch in the paste-board boxes, and then left to starve. Each lot remained together in a mass for a day or two, and then scattered and began to wander. With one or two exceptions, all of them died at about the same time. The table summarizes.

TABLE V. Length of Life in Confinement without Food, Instar I.

Lot No.	No. larvae.	Hatched.	Died.	Length Life. Days.
1.	58	9 a. m., June 11.	June 16.	5
2.	62	Noon, June 12.	June 16.	4½
3.	52	Noon, June 15.	6 a. m., June 20.	4¾
Sums	172			14.25
Averages				4.75

THE PUPA.

1. *Duration of Stage.* The few observations recorded on this point are briefly tabulated as follows:

TABLE VI. Duration of Pupal Stage, Actual Time in Soil, Different Dates. 1907.

Lot No.	No. pupae	Entered soil.	Adults emerged.	Length of time in soil.	Sums of effective temperatures, deg. Fahr.
1	40	6 p.m., July 6	12:30 p.m.July 17	10 das., 18½ hrs.	391.3°=35.5° daily average.
2	15	10 a. m., Aug. 14	10 p.m., Aug. 27*	13 das., 12 hrs.	†
3	11	5 p. m., Sep. 11	10 a. m., Sep. 25	13 das., 17 hrs.	†

* Average time: 4 at 10 a. m., Aug. 26th; 7 at 10 a. m., Aug. 27th, and 3 at 7 to 10 a. m., Aug. 29.
† Hiatus in records.

THE ADULT.

1. *Length of Life in Confinement.*

a. In pairs normally reproducing. The results obtained on this point are important, indicating as they do a very much longer adult life than formerly believed, and even going beyond the records of Tower (1906, p. 231, table 103). With Tower in normally reproducing adults, over 88 per cent. had died by the twenty-fifth day, 67 per cent. by the twentieth day. My experiments were necessarily on a much smaller scale, yet all of the pairs kept by me, and in fact all of the adults recorded in following, confined together, lived considerably over the periods recorded by Tower. For instance, the hibernated pairs of the wintering generation, captured mating at 4 P. M., May 29th, and at once confined and supplied daily with food, reproduced continuously and did not commence to die until after the middle of the following August; the male of Pair No. 2 died on August 16th, the female of Pair No. 1 on August 28th, after not quite three months, the female of Pair No. 2, not until October 6th, much over four months after capture and the male of Pair No. 1 did not die until April 6, 1908, after it had been in hibernation since July 2, 1907, emerging on February 26, 1908.

As Tower points out (1906, l. c.), these records may be due to exceptionally long-lived individuals. Table VII summarizes.

TABLE VII. Length of Adult Life in Confinement, Normally Reproducing.

Lot No.	No. individuals		Source.	Date confined, 1907.	Date of death, 1907.		Length of life, months.	
	Male	Female		Emergence	Male.	Female.	Male.	Female
I. Hiber. 1	1	1	Potato field	4 p. m., May 29	1. Ap. 6. 08.	1. Aug. 28	10+	3±
2.	1	1			2. 5 p. m., Aug. 16	2. Oct. 6.	2.6	4.25
II. Gen. 1 1	1	1	Pair No. 2 hibernated	12:30 p. m. July 17.	Hibernated, Nov. 1.	Hibernated, Nov. 1.	3.5(+?)	3.5(+?)
2	1	1						
III. Gen. 2 1	9		Pair No. 1 gen. No. 1	Aug. 26-27	Hibernated Nov. 1.	Hibernated Nov. 1.	2.2(+?)	2.2(+?)
2	4							

b. Mixed sexes, without food. A single lot of adults, survivals of 30 larvae hatching at 5 A. M., June 25th, and produced by Pair No. 2 of the hibernated individuals, and emerging from the soil in a large glass jar at 12:30 P. M., July 17th, were immediately fed and kept supplied with food until mating began on

July 22nd, when two mated pairs were removed to comprise the pairs of the first generation. The remaining 7 beetles of both sexes were fed until 10 A. M., July 27th, and then left to starve, and the soil in the jar allowed to become hard and dry. On the morning of August 11th, drops of water were added to the soil, and some of the adults drank of it; two of the beetles were then buried just below the surface of the earth. Nearly two months later, September 20th, three of the beetles died, and were found lying on the surface of the soil, which was now hard and caked, though it had been moistened from time to time. On September 25th, one adult was alive on the surface of the soil and the other three were exhumed alive; the soil was then dry and compact, but was again moistened from above on that date. The exhumed beetles again entered the soil; the one remaining above died on October 3rd. On October 17th, the three adults remaining were again exhumed alive and replaced on top of the soil, which they soon re-entered. Finally on November 1st, they were carefully examined and found in apparently good condition. They were placed on fresh, sifted soil, together with the male of Pair No. 1 of the hibernating individuals, allowed to enter it, and the jar containing them was placed in a place suitable for hibernation. They finally emerged on February 26 (1), April 7 (1) and April 11 (1), 1908, and are alive today (April 30, 1908).

Summarizing, the 7 beetles of mixed sexes lived as follows, after feeding was discontinued: 3 lived from July 27th to September 20th, or 55 days; 1 lived from July 27th to October 3rd, or 68 days; the remaining three lived at least until April 30, 1908, or over.

It is to be remarked that no reproduction occurred after food was discontinued, though a few eggs were deposited on July 23rd, during the period of feeding. As is to be expected none of the adults were observed mating either.

2. *Length of the Period of Oviposition.* By consulting table II, in which is given the number of eggs deposited by pairs of different generations in 1907, it is seen that the period of oviposition varied considerably for each generation, and was very much the longest for the pairs of the hibernated individuals. The table below makes the differences more apparent.

The period had a range of from 3 to 85 days, according to generation. Tower (1906, p. 237, table 104) gives the average length of this period for normal beetles as 30 days*, but these figures are given on the supposition that there are but two generations which reproduce, the hibernated generation and the first generation (Ib, p. 243). An average of 32 days for the period of oviposition is obtained for the three generations, providing that Pair No. 2 of generation I is ignored, there having been no amount of reproduction with this pair. I should think it fairer, however, to consider averages for each generation, as the conditions are different with each, and especially so with the hibernated pairs which have a decided advantage in regard to available time for reproduction. It is apparent from previous tables that the period of oviposition is not directly dependent upon the length of life of the adults; that is to say, the latter may be much greater than the former which does not necessarily close with the death of the female. The reproductive periods appear to be more directly dependent upon the available food supply, the season of growth of the food plant, and similar factors, and I believe the short periods of the later generations may be thus explained, a view in direct conflict with Tower's (1906) limiting physiological principle.

TABLE VIII. Length of the Period of Oviposition, Different Generations, 1907.

Generation No.	First mated.	First eggs Deposited.	Last eggs Deposited.	Length period of oviposition. days.
Hibernated—				
Pair No. 1	May 29, p. m.	a. m., June 1	2 p. m., Aug. 24	85
Pair No. 2		2 p. m., May 31	p. m., July 23	54
I.				
Pair No. 1	July 22, a. m.	3:30p.m.July 22	p. m., Aug. 6	15
Pair No. 2		By p.m., July 27	July 27	1
II				
Lot No. 1	Sept. 1	Sept. 4	Sept. 7	3
Lot No. 2		Sept. 2	Sept. 6	4

3. *Mating.* With each generation, I have recorded the number of times which I have observed mating, but undoubtedly this act took place with more frequency than the records indicate, as I was unable to watch the pairs continuously, either during the day or night, and they are by no means complete. I tabulate these observations for convenience.

Pairs were observed mating twice on the same day (Pair No. 2, hibernated individuals), usually once in the morning and once

* For special races, however, as shown by the quotations from correspondence in foregoing, the period was much longer.

in the afternoon, but on July 6, twice in the afternoon; mating was observed at the following hours of the day: 3 P. M. (May 31); noon (July 15); 4 P. M. (May 29, July 21); 2 P. M. (June 5, Aug. 6); 10 A. M. (July 22); 11:30 A. M. (July 22); 11 A. M. (July 24); 4 P. M. (Sept. 5); 7 P. M. (Sept. 5); the observations seem to show that mating occurs as frequently in the morning as in the afternoon. The table also shows the relations between mating days and period of mating to period of oviposition and number of egg-masses deposited.

TABLE IX. FREQUENCY OF MATING IN REPRODUCING PAIRS, DIFFERENT GENERATIONS, 1907.

Generation No.	Pair No.	First mating.	Subsequent matings.	Last matings.	No. of matings	Observed period of mating, days.	Period of oviposition, days.	No. egg masses deposited.
Hibernated.	1	4 p. m. May 29	May 31. June 2,3,4,5,8,9,16, 17,18,20,23,24.	June 28*	15	May 29–June 28 30 days.	85	50
	2	4 p. m., May 29	May 31. June 3,10,12,13,17, 19,20,22,24,25,28, 29. July 1,3,3,4,4,5,6,6, 9,15,18,19,21.	Aug. 6†	28	May 29–Aug. 6, 69 days.	54	34
I.	1	10 a. m., July 22.		p. m., August 1‡.	2	July 22–Aug. 1. 10 da.	15	11
	2	11:30 a. m., July 22	July 24, 30, 31	Aug. 6•	5	July 22–Aug. 6, 15 da.	1±	1
II.	Lot 1	Not observed					3	3
	Lot 2	Sept. 1, approx.	4. p. m., Sept. 5	Sept 8©	3	Sept. 1–Sept. 8. 7 das.	4	3

* Male entered earth, July 2. † Male died, August 16. ‡ Male entered earth, p. m., Aug. 5th.
• Male entered earth, a. m., August 7th. © Began to hibernate, Sept 7th.

4. *Potency of Fertilization.* No experiments were made to test this directly, but in the case of Pair No. 1 of the hibernated beetles some data have been obtained on it. Although the male left his mate on July 2nd and buried himself into the soil, the female continued to produce eggs until 2 P. M., August 24th, less than four days before her death, and 52 1-2 days after having been deserted by the male. During these 52 1-2 days, she deposited no less than 591 eggs or more than half of the total number deposited by her during the season; these eggs were deposited in 29 separate masses ranging from 1 to 60 eggs and averaging 20.3 eggs per mass; the daily rate of deposition for this mateless period was about 11.2 eggs. The eggs were tested for fertility at frequent intervals with positive results, and up until P. M.,

August 23rd, on the next to the last mass of 51 eggs. As compared to the period during which the male was present, a period of 34 days, 506 eggs were deposited in 21 masses ranging from 6 to 91 eggs and averaging about 24 eggs per mass, and with a daily rate of deposition of about 14.9 eggs; there is apparently some difference, as the daily rate of deposition fell after the male left, though his absence did not seem to matter very much. However, it is idle to speculate about a single observation of this kind. The simple fact is that the female continued to deposit fertile ova for many days after the male left.

It is well to note in this connection, the fact that oviposition in Pair No. 2 of the hibernated beetles stopped quite early, on July 23rd, 24 days before the death of the male and 74 days before that of the female; the female of this pair therefore lived for fifty days after the death of the male but deposited no eggs. I fail to account for the lack of production during this period, and merely mention the fact in order to show the singular difference in behavior between the two pairs. In Pair No. 1 of the first generation, both sexes disappeared beneath the soil at the same time, whereas in Pair No. 2 of the same generation, the male entered the soil fifteen days before the female, but again no deposition occurred after his desertion, and in this case but very little before.

5. *Number and History of Generations Reared in the Laboratory.* At 4 P. M., May 29th, 1907, as previously stated, two normal pairs of this insect were captured *in copula* in a potato field a quarter of a mile west of New Richmond, Ohio, and these were at once brought to the laboratory and confined, each pair separately, in a suitable glass jar containing moist soil and covered with cheese-cloth. They were kept constantly supplied with fresh potato foliage and soon began to produce a continuous series of eggs as shown in table II. These pairs were evidently hibernated individuals and they constituted the parents of the first generation.

Eleven adults emerging from the soil at 12:30 P. M., July 17th, being descendants of Pair No. 2 of the hibernated individuals (*cf.* table X) were confined together with food on that date, and on July 22nd, two of the pairs observed mating at 10 and 11:30 A. M., respectively, were transferred to separate jars; these constituted the parents of the second generation. Pair No. 1 of these two (*cf.* tables I and II) deposited 21 eggs which hatched at noon, August 3rd, the resulting thirteen adults emerging from the soil at 10 A. M., August 26th and 27th, and constituting the parents of the third generation, which unfortunately was not carried through to the adult stage, though larvae were obtained from the 96 eggs deposited by them. The following schema and table represents graphically these generations reared in confinement. Other relative data are given in connection with a preceding table (table II).

Schema of Generations Reared in the Laboratory, New Richmond, Ohio, 1907.

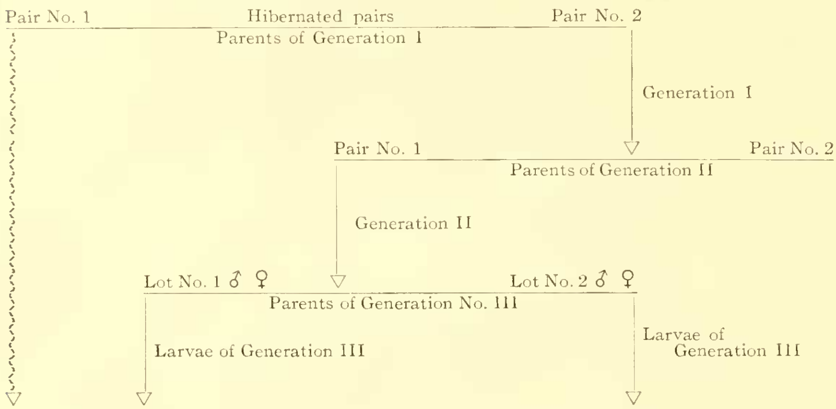


TABLE X. Generations Reared in the Laboratory, New Richmond, Ohio, 1907.

Generation No.	Eggs deposited.	Adults out.	Length of cycle. Days.	Length of cycle. Hours.	Effective temperature, sum.
I.	1 p. m., June 20th.	12:30 p. m., July 17.	26	23½	897.2° Fahr.
II.	1 p. m., July 29.	10 p. m., Aug. 26.	28	9	*
III.	Sept. 2–4.	Not reared to maturity.			

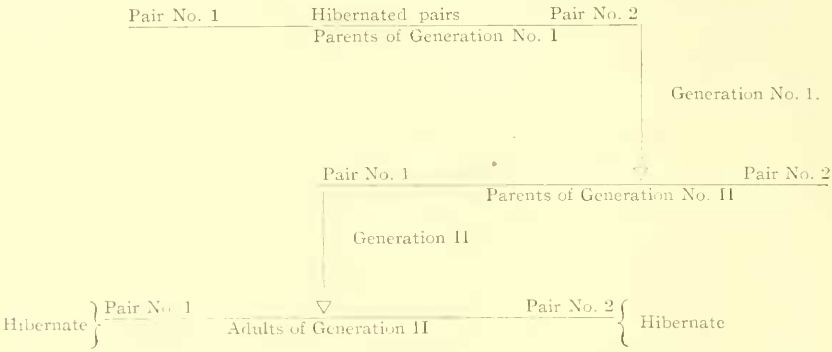
* Not obtained.

It is interesting to note in connection with the third generation, that although in itself not reared to maturity on account of the lack of opportunity, yet an almost parallel cycle was obtained from a series of 13 larvae hatching at 8 A. M., August 30th, from eggs deposited by Pair No. 1, hibernated individuals or parents of the first generation, and coming to maturity at 10 A. M., September 25th; this generation of larvae was not more than four or five days earlier than the third generation. There can be no doubt that the parents of the third generation (or the adults of the second generation) were willing to reproduce to some extent before going into hibernation, and there appears to be no reason why the larvae so produced should not have reached maturity with ease. It seems perfectly reasonable to me to have reared at least three, if not four, complete cycles of this insect during the season, if the breeding had been started about the first of June, instead of more than three weeks later, as unfortunately was the case.

Although the foregoing looks clear enough to me, but because of the conditions being those of confinement and on so small a scale, I approach with caution the statements of Tower (1906, p. 243), on the number of generations in Leptinotarsa decemlineata. Quoting directly from this work, he says: "The number of generations in Leptinotarsa each year, in both temperate and tropical latitudes, is a remarkably constant character, and might well be used as a generic differential. As far as I know, the number in all of the species is limited to two. Thus, there are two generations throughout the range of decemlineata, although Lugger has recorded three in Minnesota, and others have supposed that there may be three in the southern United States. I have not, however, been able to get decemlineata to breed more than twice in a season without a period of hibernation or aestivation. In the spring decemlineata emerges from the ground, and after a period of feeding, during which the germ-cells are also maturing, it breeds and lays the eggs for the first generation. These are usually all deposited at about the same time, but there are always for a month or more some individuals that are laying eggs, and of course the larvae and imagines resulting from these eggs which are last laid are much later in maturing than are the majority of the population. The first brood, on emergence, feeds for a few days, and then deposits the eggs for the second generation. The majority of these eggs hatch late in the summer, and after the animals feed and fly around for a month or more they burrow into the ground, and there hibernate until the following spring. The second generation does not develop the germ-cells nor show any reproductive activity until after it has passed through a period of hibernation or aestivation. Beetles are found breeding even late in the autumn, but these are the belated individuals of either the first or second generation. As far as I can discover, the life cycle in this species is that given above." Further down on the same page, he says: "The species in the genus are therefore double-brooded, the second brood undergoing hibernation or aestivation before reproductive activity is resumed." And again on page 244: "In all the species of the genus the only difference between the two generations is that the second does not develop the germ-cells until after a period of rest, while the other develops them at once, or soon after emergence."

Although these statements are not very clear to my mind, because of certain ambiguities*, yet the following schema represents my interpretation of Tower. This schema, for the convenience of comparison with the one in foregoing (p. 168), is based on two pairs for each generation.

SCHEMA REPRESENTING AN INTERPRETATION OF TOWER'S GENERATIONS OF DECEMLINEATA.



By comparing the two schemata, it is at once apparent that a portion of a third generation is represented by my observations, and this is clearly in disagreement with the reiterated statements of Tower, to the effect that the adults of the second generation do not breed, or even show signs of breeding, before a period of hibernation or aestivation. This fact would not be considered of much importance, owing to the laboratory conditions and meagreness of the observations, were it not for the statement made by Tower (*Ib.*, p. 246) that "In decemlineata I have not by any process been able to prevent this preparation for hibernation in beetles which should normally hibernate." The mating and reproduction of the adults of the second generation are therefore very,

* For example, the two successive sentences on page 243: "The *second generation does not develop the germ-cells nor show any reproductive activtiy until after it has passed through a period of hibernation or aestivation.*" and "Beetles are found *breeding* even late in the autumn, but these are the belated individuals of either the first or *second* generation." It is clearly implied in the last sentence that beetles of the second generation breed, which flatly contradicts the declaration of the first; there exists a confusion of terms both here and elsewhere in the discussion. The italics here are mine.

very exceptional, according to Tower, for it is implied that he was unable to find such either under a great variety of natural habitats, or under all possible laboratory and experimental conditions. Hence it seems all the more strange that these phenomena should occur from the first under ordinary laboratory conditions, in a breeding experiment which was purely incidental.

But Tower does not give all of the matter concerning this question together, and it is in the later portion of his work that we find the most pertinent data on it. This is in relation to the production of special races by breeding under controlled conditions.

If a race can be considered the same as its species, Tower practically admits that there *may be* on occasion, more than two annual generations, and thus contradicts himself, for he says on page 251: "Closely associated with the hibernation of the second generation in these beetles is the quiescent or resting period in the cycle of the germ plasm. That is, the germ cells do not develop at all in the autumn, but remain as oocytes or spermatocytes, which are relatively few in number, until the following spring after emergence from hibernation, when they develop rapidly. This period of inactivity in the reproductive elements has been regarded as due to some inherent necessity for rest in the germ plasm. However, it does not seem to me to be of this nature; it is rather in the nature of a very deep-seated adaptation, like aestivation, which has been developed and retained not only in this genus, but also in the whole insect phylum, for the purpose of enabling them to pass successfully over the season of the year when unfavorable conditions of existence are most apt to occur. In my experiments a race arose suddenly in which there were five generations, and then a period of rest, and then five more, and so on." From this quotation, it is apparent that Tower obtained five seasonal generations with what he calls a *race* of the *species* decemlineata. I believe, however, that the distinction is justifiable because the appearance of the individuals with five annual cycles, under controlled conditions in confinement, was relatively scarce, abnormal, and they had racial characteristics.

Again, Tower (*Ib.*, pp. 280–281) gives another illustration of the occurrence of more than two annual cycles: "A variation which arose from decemlineata obtained at McPherson, Kansas, appeared in my culture in June, 1904. This was rubrivittata, of striking form and coloration, of which there was a single male. The parents, which were collected in July, 1903, near McPherson, Kansas, were sent to Chicago and reared in a second generation which was normal; they hibernated until May, 1904, and then emerged, reproduced, and among the progeny was this single male rubrivittata. This male was crossed with a female decemlineata from Chicago, and gave a hybrid brood intermediate in character between the parents. Three males and one female of this lot escaped the general extermination of my experiments in July, and were carried over into 1905, giving after transfer to Mexico in March, 1905, a Mendelian splitting into typical rubrivittata and hybrid forms (text—fig. 22). These were separated and reared. The pure cultures of rubrivittata showed as a result a new character, namely, that its life-history as well as less important characters were changed, there being three generations in its yearly cycle instead of two, as in the parent species, and in all of the species in its immediate ancestry.

"This change in the life cycle from hibernating in every second generation, as do most of the species in the genus, to hibernating in every third, is striking and significant, the three generations being gone through in about the same time as the two of the parent species. The cultures with rubrivittata demonstrate clearly that *changes in physiological characters can take place rapidly, as do changes in structure*, and that these changes may alter not only unimportant characters, but most fundamental ones as well. We shall have occasion to consider a similar case even more striking and interesting in a later portion of this chapter."

The remaining case referred to in the last sentence of the quotation just ended (from pp. 280–281) is now given for convenience and in order to place all of them as near together as possible; space does not allow of its being given in full, and I give a brief history of it until the variation appears. Beetles emerging from hibernation in May, 1901, (Tower, Ib., p. 288) were reared until May, 1902, under normal conditions without extreme variation. They were then divided into two lots and subjected, one to hot and dry conditions, and the other to hot, dry and low pressure conditions. The first is considered.*

"From the apparently pure stock, 7 males and 7 females were in May, 1902, subjected during the first half of their reproductive

*The other lot appears to have been ignored, though a statement is made to contrary (p. 288).

period to hot, dry conditions, during which time they laid 409 eggs. For the latter half of the reproductive cycle they were kept in normal conditions, laying 840 eggs, From the 409 eggs developed and laid in changed surroundings I obtained 64 adults, as follows:

"A(1) Normal (apparently) decemlineata, 12 males, 8 females; (A 2) L.pallida, † 10 males, 13 females; (A 3) L. immaculothorax,* 2 males, 3 females; and (A 4) L. albida,† 9 males, 7 females. These four lots were separated and reared, with the exception of pallida. The 840 eggs laid in normal conditions gave 123 normal decemlineata, and these I designated B.

"The Lots A1 and B were reared side by side in the following generations and both gave normal beetles as far as could be determined, but as the period for hibernation approached, those of A1, instead of going deep into the ground, as did B and as is normal, aestivated on top or close to the top. The Lot B went into hibernation in September, A 1 in late October and early November. In January (January 2) Lot A 1 emerged from aestivation and began breeding, giving a brood, part of which hibernated and part continued breeding for five generations, then hibernated, and then emerged and bred through five more generations. These hibernated again, and in the fourth generation of the third cycle of five generations were killed in July, 1904. These successive generations were all reared under exactly similar conditions, nor‡ was there any conscious selection practiced. Free interbreeding in each lot was allowed. The general result is expressed in text-figure 26.

"This race with a cycle of five generations is of great interest, showing the profound modification resulting in the reproductive cycle. None of the beetles of the lineata group, to which this beetle belongs, have more than two, or rarely three generations per year, and there are none known in the genus that have over three. Clearly, then, this race with five generations in each cycle is quite a new character in the genus, and was, as far as discovered, constant|| from the start, showing in the fourteen generations no tendency to revert to the parental standard. As far as I can discover, this case can be explained only as the direct response of the germ plasm to the extreme stimuli used in the experiment.

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* L. decemlineata pallida Tower; L. decemlineata immaculothorax Tower.
† L. decemlineata albida Tower. (Cf. p. 92).
‡ Beginning p, 289.
|| Beginning p. 290.

It seems impossible to account for the condition produced upon the basis of a latent character of this kind somewhere in the ancestry of this genus. Moreover, all of the beetles in this experiment had an equal chance to be accelerated by the conditions of existence, so that this factor could not by any stretch of the imagination be held to account for the development of this change. As far as is known, the only changed factor in this experiment was that used in the third generation upon the 7 males and 7 females for three-fifths * of the reproduction period†. From the germ cells subjected to stimuli this race arose, but not at once, two generations being necessary in which to disentangle the changed character from the non-modified decemlineata."

Therefore, in order to agree with Tower's results, the production of eggs by the adults of the second generation of the beetles reared by me in 1907, will have to be considered as due to some abnormal stimulus present during maturization of the reproductive elements, or as a racial characteristic; but it seems to me that either supposition is improbable, because of the few generations through which the beetles had been inbred. Tower's evidence is poorly presented, without reservation or limitation in places, it is contradictory and it is to be regretted that in a great many places he has contented himself with giving general statements without the accompanying data as evidence; conditions that produce controversy instead of establishing fact. When his evidence is sifted, however, it is found that the following facts are indicated as being true:

1. That decemlineata, when bred in confinement in normal environments, in all cases goes through but two annual cycles the second of which hibernates before reproduction.
2. That decemlineata, when bred in confinement through more than three generations in abnormal controlled environments, in the great majority of cases goes through but two annual cycles, as in normal beetles.
3. That the species, when bred in confinement for more than three generations in abnormal controlled environments, exceptionally may go through as many as five annual cycles, this physiological variation being correlated with morphological variations of racial value, produced suddenly.

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* One-half? (Cf. Statement of conditions, p. 288).

† A foot-note follows here, which is omitted in the quotation.

These being true, the facts recorded by me in regard to the production of a third generation are indeterminate in value, and exceptional in nature, for apparently there were neither abnormal environmental factors present, nor variation tending to racial evolution. It would be highly interesting and important, therefore, if more of this breeding of annual cycles under normal or natural conditions, was carried on, in two similar series, in the laboratory and in the field under as natural conditions as possible. The question of the number of annual generations of decemlineata freely breeding in nature has not been determined yet by actual experiment, or at least such are not recorded with cold evidence, and Tower (1906) has not decided it, but perhaps left it in a more controversial position than ever; he has, however, made a long stride in the direction of settlement.

But looking at it from another standpoint, I believe that Tower (1906), although in the proper place (Ib., Chapter IV, p. 243) not presenting definite data upon which his conclusions are based, has the best right to be followed because of his large experience with breeding the species in question. As I understand it, he speaks of normal, average conditions in nature, and I do not know that any direct or definite evidence has yet been presented to show that the species is more or less than double-brooded in nature, and Tower certainly has had enough experience with decemlineata in nature to speak with authority on the subject. There are certainly as many as two annual cycles, if repeated laboratory experiments can be at all trusted, and Knab's (1908) recent contention that there is but one, based on the systematic relations of the beetle is not tenable, and to my belief, wholly without basis or foundation for advancement. The question now is: Are there but two, or more than two annual cycles with decemlineata; or, in other words, does the second generation reproduce? This does not concern any other species of Leptinotarsa or species of Calligrapha, Lina or Gastroidea, but the sole species under consideration.

I have myself recorded reproduction by a first reared generation of Gastroidea cyanea Melsheimer (Girault, 1908, p. 8), making two annual cycles possible, but I can't see what the number of generations in closely related genera of the family has to do with the question at issue, especially since the question with these genera is not established. It was also going beyond the point to say that the seeming double-broodedness of decemlineata "may be due to the difference in time of emergence from hibernation of different individuals," for Tower (1906, pp. 202, 266–267, *et al.*) gives evidence by direct breeding, thus throwing this out of the question.

And summing up, all of this discussion but again calls attention to the crying need for facts. We want tangible evidence, not inductions, views, opinions, or claims.

6. *Behavior of Adults in Confinement; Hibernation.* The pairs kept in confinement, normally reproducing, behaved in some instances peculiarly; this was especially noticeable in the case of the male of one of the hibernated pairs. In pair No. 1 of the hibernated beetles, the two sexes behaved normally until on July 2nd, the male disappeared and was found on the morning of July 3rd buried beneath the soil; it was replaced on the surface but again went beneath after several hours had elapsed. It was exhumed again at 3 P. M., July 6th, and was again beneath within the following hour, and the same fact holds for 10 A. M., July 9th. It was re-exhumed at 5 P. M., July 15th; 3 P. M., August 5th; August 19th (A. M.); 11 A. M., August 28th, September 2nd, October 17th and 23rd, each time re-entering at once and paying no attention to the female or food. On November 1st, it was again disturbed and given fresh quarters for hibernation; it finally emerged (it was kept in a cold room) on February 26th, 1908, and died on the following April 6th; it had apparently hibernated during two winters, that of 1906–1907 and 1907–1908.

The male of pair No. 2 of the hibernated pairs behaved normally until its death at 5 P. M., August 16th. The male of pair No. 1 of the first generation entered the soil P. M., August 5th, and the female P. M., August 7th; both were exhumed alive at 10 A. M., August 19th and replaced on the surface with fresh food which they ignored and re-entered the soil. They were exhumed alive on November 1st and liberated. The male of pair No. 2 of this generation entered the soil A. M., August 7th and was exhumed at 10 A. M., August 19th; it at once re-entered. On August 22nd, the female of this pair entered the soil, and at 10 A. M., September 2nd, both were exhumed alive and replaced on the surface of the soil into which they soon disappeared. On October 17th and 23rd, the exhumation was repeated with similar results; they were dug up alive and liberated on November 1st, 1907, with the other pair. In Lot No. 1 of the second generation, 2 entered the earth on September 7th, and on the 9th of the same month the remaining beetles entered (between the two dates, their food had of necessity been neglected and had wilted); on October 17th the seven were exhumed alive and placed on the surface of the soil into which they had disappeared within an hour. They were exhumed alive and liberated with the others on November 1st. The adults of lot No. 2 of this generation entered the soil together on September 12th, and were undisturbed until liberated on November 1st, all in good health and hibernating.

Even when entering the conditions of hibernation in the warm summer months, it seems almost impossible to break into the habit and induce the beetles to feed or mate. Their physiological condition must be profoundly modified in this state, as has been found to be the case by Tower (1906, p. 245ff.)

General Field Conditions, Supplementing the foregoing.

I was unable to watch the beetles in the field at all closely, so that but a few fragmentary observations were made; these will serve, however, to give some idea of the general conditions prevailing with the species in the late summer of 1907. It should first be stated that the season of 1907 was very abnormal, in that the spring was at first far advanced in March, it then being very warm, and later in April retarded by a cold wave which killed young plants and newly set fruit or the far advanced fruit buds; planting was therefore much delayed in many cases, but data kindly gathered for me by the Reverend Mr. C. L. Chapman of New Richmond, showed that the potato crop was at least an exception. The following table compiled by Mr. Chapman shows the relative times of planting and harvesting of the potato crop in the vicinity of New Richmond, Ohio, for the three seasons past.

TABLE XI. Relative Times of Planting and Harvesting Potato, 1905–1907.

Year.	Preparation of Soil.	Time of General Planting.	Time of Harvest.
1905.	March 27–April 20.	March 30–April 30.	September 1–October 1.
1906.	April 1–April 20.	April 13–20	July 20–August 31.
1907.	March 25–April 15.	March 29–April 20.	September 30–October 10.

From the table, it appears that the crop remained in the field somewhat later than usual in 1907, and this would apparently have some bearing on the breeding of the beetles.

On September 2nd, 1907, it was noted that adults were still mating in numbers, and larvae were also abundant; this observation made in a single field. On the 6th of the same month in the same field adults and large larvae were numerous, but eggs were relatively scarce; several egg-masses were found, however. On September 25th, a long search in this field failed to find either eggs or larvae, and but very few adults and these were extremely restless. I find unfortunately that these fragments are all that were recorded, and they leave much to be desired.

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